In the Specification

Please amend the paragraph beginning at page 8, line 18 as follows:

Traditional method for rAAV production is: co-transfecting rAAV vector plasmid and helper plasmid containing rep-cap gene into the cell, followed by helper virus infection, such as adenovirus or herpes simplex virus. After 2-3 days, the recombinant AAV (rAAV) and the used adenovirus or herpes simplex virus could be harvested from culture supernatant as well as pathologic cells. Adenovirus and herpes simplex virus could be deactiviated by heat treatment (under 55□°C for 30 minutes to 2 hours) without prejudice of AAV reactivity.

Please amend the paragraph beginning at page 21, line 23 as follows:

HSV 1 genome segments loaded in cos6 and cos56 cosmids each have a XbaI single enzyme cutting site, respectively locating whithin nonessential genes UL2 and UL44, generally useful for inserting exogenous genes. After XbaI enzyme cutting, gene segments r2c1, r2c3, r2c4, r2c5 and r2c6 are inserted into XbaI site of cos6 to construct recombinant cosmids cos6-r2c1ΔUL2, cos6-r2c3ΔUL2, cos6-r2c4ΔUL2, cos6-r2c5ΔUL2, cos6-r2c6ΔUL2, respectively (generalized as cos6-rXcYΔUL2, see Fig.6). Any one of cos6-r2c1ΔUL2, cos6-r2c3ΔUL2, cos6-r2c4ΔUL2, cos6-r2c5ΔUL2, cos6-r2c6ΔUL2 is then respectively equimolarly mixed with cos14, cos28, cos48, cos56, and, removing cos framework with PacI enzyme cutting and cotransfecting BHK-21 cells with liposome, so that five HSV1 segments undergo homologous recombinations in cells and respectively give such recombinant viruses as HSV1-r2c1, HSV1-r2c3, HSV1-r2c4, HSV1-r2c5, HSV1-r2c6. Five days later, cells begin exhibit pathology, collecting the culture supernatant after complete pathology of the cells, then centrifugating at 2000r/min for 5 min, and dividing the supernatant in aliquots for storage under -20□°C. The probability of producing recombinant HSV1 virus containing target DNA segment is as high as 50~100%. It is easy to pure recombinat virus through further plaque screening.

Please amend the paragraph beginning at page 22, line 10 as follows:

Similarly, it is also feasible to obtain recombinant viruses having the same functions as the recombinant viruses HSV1-r2c1, HSV1-r2c3, HSV1-r2c4, HSV1-r2c5, HSV1-r2c6 by respectively inserting such gene segments as r2c1, r2c3, r2c4 and r2c5 into XbaI site of cos56. The preparation process is as follows: after XbaI enzyme cutting, gene segments r2c1, r2c3, r2c4, r2c5 and r2c6 are respectively inserted into XbaI site of cos56, in order to respectively construct recombinant cosmids cos56-r2c1ΔUL44, cos56-r2c3ΔUL44, cos56-r2c4ΔUL44, cos56-r2c5ΔUL44, cos56-r2c6ΔUL44 (generalized as cos56-rXcYΔUL44, see Fig.7). Any one of cos56-r2c1ΔUL44, cos56-r2c3ΔUL44, cos56-r2c4ΔUL44, cos56-r2c5ΔUL44, cos56-r2c6ΔUL44 is then respectively equimolarly mixed with cos6, cos14, cos28, cos48, and, removing cos framework with PacI enzyme cutting and cotransfecting BHK-21 cells with liposome, so that five HSV1 segments undergo homologous recombinations in cells and respectively give such recombinant viruses as HSV1-r2c1, HSV1-r2c3, HSV1-r2c4, HSV1-r2c5, HSV1-r2c6: five days later, cells begin exhibit pathology, collecting the culture supernatant after complete pathology of the cells, then centrifugating at 2000r/min for 5 min, and dividing the supernatant in aliquots for storage under -20□°C. The probability of producing recombinant HSV1 virus containing target DNA segment can also be as high as 50~100%. It is easy to pure recombinat virus through further plaque screening.

Please amend the paragraph beginning at page 26, line 14 as follows:

4) Solid polyglycol 8000 is added to the supernatant to a final concentration of 6~12%, accompanied by stirring for dissolution. The mixture is disposed at 4□°C for 1 hour to overnight. The mixture is then centrifugated at 12000g for 10~15min. Afterwards, the supernatant is decanted into another clean conical flask, letting the supernatant draining away as much as possible. The precipitation is then dissolved with an appropriate amount of PBS^{2+}, and DnaseI and RNase are added to digest residual DNA and RNA aside from AAV particles. Equiareal chloroform is added for extraction, and the mixture is centrifugated at 12000g for 5min. The upper aqueous phase is carefully taken out under sterile operation, and transferred into a sterile pipe. The solution is the very concentrated and purified rAAV solution.

Please amend the paragraph beginning at page 30, line 25 as follows:

Example 1-1 Construction of cos6-r2c1□△UL2

AAV-1 was used as the template, and the corresponding cap1 (AAV-1) was amplified using PCR method (for primers refer to Primer Sequences 1 and 2). The reaction conditions were 30 circles of 94□°C for 30 sec, 55□°C for 30 sec and 72□°C for 3 min. A PCR segment of 2210bp, cap1 was obtained. After double digesting with restrictive enzymes KpnI+XbaI, cap1 was ligated with rep 2 of 1721bp, which was cut from pSSV9 with KpnI+XbaI. The ligation product was inserted into XbaI site of plasmid pGEM-p3zf(+) (Promega Co.) to form p3zf-r2c1 plasmid. After digested from p3zf-r2c1 plasmid with XbaI, r2c1 (of around 4347bp) was inserted into XbaI site of cos6. As a result, cos6-r2c1□△UL2 was obtained.

Please amend the paragraph beginning at page 31, line 12 as follows:

Cos6-r2c1□△UL2 was mixed at equal molar ratio with five cosmids such as cos14, cos28, cos48, cos56 and the like. Then, the cos framework was removed by PacI enzyme (no need of remove the cos framework by isolation). After that, the mixture was extracted with phenol, phenol/chloroform (1:1) and chloroform respectively. The supernatant was removed, and DNA was precipitated using 2.5 times anhydrous ethanol. 20ul of lipofactamine (GIBCO BRL) and 10 ug of DNA were used to co-transfect an 80% confluent BHK-21 cell (about 2×10^6) according to the instructions, then homologous recombination of the 5 HSV-1 segments occurred inside the cells and accordingly HSV1-r2c1 recombinant viruses were generated, respectively. After 24h of transfection, the cell was cultured in 1640 medium containing 2% FBS at 37□°C. The medium was changed every day. After 5 days, the cell began exhibit pathology. The supernatant of the medium was collected after complete pathology. The supernatant was centrifugated at 2000 r/min for 5 min. Then, the supernatant was aliquoted and stored at -20□°C. Thus obtained recombinant viruses were plaque-purified twice so that pure HSV1-r2c1 recombinant viruses were prepared.

Please amend the paragraph beginning at page 32, line 9 as follows:

AAV-1 and adenovirus 5 were used to infect 293 cells. The cell was freezed and thawed 3 days later, and then was centrifugated (5800g) for 30 min, and purified with CsCl as described in

J.V.1997, 71: 8429-8436. The above-mentioned AAV-1 viruses were treated with 0.1% SDS and protease k of 0.2 mg/ml at 37□°C for 3h, then was extracted with phenol/chloroform twice and extracted with chloroform once. Sodium acetate and alcohol were added to precipitate DNA. After deposition, the DNA was resuspensed by TE(PH8.0)at 95□°C for 5 min and then treated in 0.3-1.0 M NaCl at 50-60□°C for 2h until the double-strands was annealed. Qiaex IIgel extraction kit (Qiagen) was used to purify the AAV-1 DNA band of about 5 kb running on agarose gel, then the ends of the DNA band were blunted with Klenow large segment, following addition of a linker of XbaI Linker (dCTCTAGAG) and purification, it was cut with XbaI, loaded in XbaI site of pGEM-3zf (a product of Promega Co.) and amplified in E.Coli DH5α Max Efficiency. After the single clones were picked out and the plasmid was extracted, those clones containing complete AAV-1 genomes were screened via restrictive enzyme and the rep2 probe method. Then, the plasmid was used to transfect BHK cells, and 24h later the cells were infected by HSV-1. 2 days later, small molecular weight DNAs outside the cell chromosome were extracted using Hirt method, cut with DpnI enzyme, transferred by Southern blotting and hybridized using a rep probe, and the integrity of genomes was indicated by a band of Dimer using a monomer. After all those, pAAV1 was obtained. pAAV1 was double digested with Eco47-3 and NcoI to recove the vector plasmid segment containing AAV-1 ITRs, which was blunted with T4 DNA polymerase; the resistance gene neo^r was cut with Bgl II and SmaI from pSV2neo of Promega Co., recovered and blunted with T4 DNA polymerase, and then loaded in the vector plasmid segment containing AAV-1 ITRs. Then, pSNAV-1 was cut with XhoI and BamHII enzyme to recover the CMV-PolyA segment, which was blunted with T4 DNA polymerase, and loaded in the vector plasmid segment containing AAV-1 ITRs. Accordingly, a recombinant plasmid pSNAV-N1 containing AAV-1 ITR elements was obtained.

Please amend the paragraph beginning at page 33, line 13 as follows:

BHK/pSNAV-GFP cells were infected with HSV1-r2c1. After cells exhibited pathology (36-72h), the cell was repeatedly frozen and thawed for 4 times. Thus, the cell lysis solution contained rAAV/r2c1-GFP and helper viruses HSV1-r2c1. A low-speed centrifugation was carried out to remove the cell debris, and then the lysis solution was treated at 56□°C for 30 min so that the helper virus HSV1-r2c1 was deactivated. Accordingly, rAAV/r2c1-GFP having the serotype of AAV-1,

which could be used to infect a cultured mammal cell *in vitro* or *in vivo*, was obtained from the supernatant of the cell lysis solution.

Please amend the paragraph beginning at page 34, line 1 as follows:

pSNAV-GFP were introduced into BHK-21 cells (purchased from ATCC and cultured in RPMI1640 medium containing 10% FBS at 37±°C) using transfecting agent Lipofectamine (produced by GIBCO BRL Co), and then the cells were selectively cultured for 10-15 days in medium with G418 of 800 ug/ml. As a result, vector cells BHK/pSNAV-GFP for mixing cell clones were obtained. Then, after being enlarged propagation, the vector cells were cultured in four 35 cm^2-square glass culture vessels; after confluent (about 8×10^7 cells), the vector cells were digested with trypsin, inoculated into roller bottles (110×288mm) followed by cultivation under a low-speed (1 rpm) rolling at 37±°C. The volume of the medium was 200 ml per roller bottle. 3 days later, the cells in the roller bottle were digested with trypsin and then introduced into 5 roller bottles for enlarged cultivation. After confluent (about 2×10^9 cells), the medium was poured out and 5-10 ml of helper viruses HSV1-r2c1 (MOI = 0.5-2) was added in prior to the 1-2 hr of adsorption of the viruses at a low speed (1 rpm). Then, a serum-free 1640 medium (200 ml per roller bottle) was added in to culture the cells at 37±°C at a low speed (1 rpm). After pathology complete, cells could be shedding easily, the bottle tops were closed tightly before being violently vortexed until all the cells attached to the bottle side were eluted into the medium. After that, the cultures in the 5 roller bottles were pooled and gathered, and after the estimation of their volumes, they were divided into erlenmeyer flasks (specification: 500 ml), 250 ml per flask, for the next purification step.

Please amend the paragraph beginning at page 34, line 20 as follows:

Continuing the above Example. 25 ml of chloroform (10:1 v/v) was added to each erlenmeyer flask, which was placed in a shaker at 37±°C for 1～1.5 hr under violent shaking before being taken out and sit at a room temperature for 10 min. DNase and RNase were then added in until the final concentration was 1 μg/ml. Then, after the mixture was blended gently until it became homogeneous, it was being digested at a room temperature for 30-60 min. Solid sodium chloride was added to a final concentration of 1 mol/L under shaking for dissolution. After that, the mixture was

centrifugated at 12000 rpm at 4□°C for 15 min before the upper water phase was extracted and the chloroform and deposits were discarded. Subsequently, PEG8000 was added in until the final concentration was 10% (w/v), and shaken to be dissolved. Then, the mixture was sit at 4□°C overnight before being centrifugated at 11000 rpm at 4□°C for 15 min. The supernatant was poured into a clean container (centrifuge tubes were turned upside down on the absorbent paper so that the supernatant could be drained to the full). After that, 5ml PBS^{+} buffer was used to beat upon, elute and gather the deposits attached to the tube bottoms and tube sides of all the centrifuge tubes. Then the gathered deposits were divided into plastic centrifuge tubes (specification: 1.5 ml), 0.6 ml per tube, before being extracted by chloroform in an equal volume. The extracted mixture were centrifugated at 12000 rpm at 4□°C for 5 min before the upper water phase was carefully extracted under antiseptic operations and then transferred into an antiseptic tube. The obtained liquid was just a condensed and purified rAAV/r2c1-GFP viral solution, of which the volume was 200 times condensed than its initial volume.

Please amend the paragraph beginning at page 35, line 20 as follows:

Continuing Example 1-7. The titer (particles/ml) of the rAAV/r2c1-GFP viruses in the purified viral solution was detected by Dot-Blotting Method using the Digoxigenin-Labeled (Boehringer Mannhein kit) GFP probe. 10 ul of the purified viral solution was diluted 1:10 with PBS^{2+} buffer. DNase and RNase were added in until the final concentration was 1 μg/ml. After the mixture was being digested at 37□°C for 1 hr and treated in boiling water-bath for 5 min, it was placed in ice bath and then diluted 1:10 with a dilution buffer before dot-blotting (1 ul/dot). Later, the membrane was baked at 120°C for 30 min, the prehybridization was conducted at 68°C for 1 hr followed by probe dot-blotting at 68°C overnight. Afterwards, the membrane was washed and developed. Results: dots 1-4 were definitely positive while dot 5 was weak positive. Supposing the sensibility of detection of DNA by Dot-Blotting Method was 10^{6} molecules, it could be calculated that the viral titer=$10^{4-5}\times10^{6}\times10\times1000=10^{14-15}$ particles/ml.

Please amend the paragraph beginning at page 36, line 6 as follows:

At 37±°C, a 10% FBS-containing RPMI1640 medium and 5% CO_2 were used to culture HeLa cells, which were then seeded in a 24-well plate (5×10^5 cell/well). After cultivation overnight, the medium was aspirated; 10 ul of purified rAAV/r2c1-GFP viral solution was diluted 1:10 to 1 ml, then each well was added with 0.5 ml of viral solution with different dilutions and was cultured at 37±°C for 1 hr; subsequently, each well was added with 50 ul of Ad-5 (MOI=5) and 0.5 ml of medium. After 36 hr of cultivation at 37±°C, green fluorescent cells could be observed under an inverted fluorescence microscope. After the number of green cells (represented by "n", wherein $10<n<100$) in a certain well had been counted, the viral titer of rAAV/r2c1-GFP could be calculated as follows: $n\times$Times of Dilution$\times1000/5=n\times10^9\times200=2n\times10^{11}$ TU/ml. Thus, it could be estimated that the infectious titer of the rAAV/r2c1-GFP virus was between 2×10^{12} TU/ml and 2×10^{13} TU/ml.

Please amend the paragraph beginning at page 37, line 17 as follows:

Example 2-1 Construction of cos6-r2c3±△UL2

AAV-3 was used as the template, and the corresponding cap3 (AAV-3) was amplified using PCR method (primers refer to Primer Sequences 3 and 4). The reaction conditions were 30 circles of 94±°C for 30 sec, 55±°C for 30 sec and 72±°C for 3 min. A PCR segment of 2040bp, cap3 was obtained. After double digesting with restrictive enzymes XhoI and XbaI, cap3 was ligated with rep 2 of 2040bp, which was digested from pSSV9 with XhoI and XbaI. The ligation product was inserted into XbaI site of plasmid pGEM-p3zf(+) (Promega Co.) to form p3zf-r2c3 plasmid. After cut from p3zf-r2c3 plasmid with XbaI, r2c3 (of around 4287bp) was inserted into XbaI site of cos6. As a result, cos6-r2c3±△UL2 was obtained.

Please amend the paragraph beginning at page 38, line 5 as follows:

Cos6-r2c3±△UL2 was mixed at equal molar ratio with five cosmids such as cos14, cos28, cos48, cos56 and the like. Then, the cos framework was removed by PacI enzyme (no need of remove the cos framework by isolation). After that, the mixture was extracted with phenol, phenol/chloroform (1:1) and chloroform, respectively. The supernatant was aspirated and collected, and DNA was precipitated using 2.5 times anhydrous ethanol. 20 ul of lipofactamine (GIBCO BRL)

and 10 ul of DNA were used to co-transfect 80% confluent BHK-21 cells (about 2×10^6 cells) according to the instructions, then homologous recombination of the 5 HSV-1 segments occurred inside the cells and accordingly HSV1-r2c3 recombinant viruses were generated, respectively. After 24h of transfection, the cell was cultured in 1640 medium containing 2% FBS at 37□°C. The medium was changed every day. After 5 days, cells began to exhibit pathology. The supernatant of the medium was collected after complete pathology followed by centrifugating at 2000 r/min for 5 min. Then, the supernatant was aliquoted and stored at -20□°C. The obtained recombinant viruses were plaque-purified twice so that pure HSV1-r2c3 recombinant viruses were prepared.

Please amend the paragraph beginning at page 38, line 20 as follows:

AAV-3 and adenovirus 1 were used to infect 293 cells. 3 days later, the cells were frozen and thawed, and then were centrifugated (5800 g) for 30 min, and purified with CsCl as described in J.V.1997, 71: 8429-8436. The above-mentioned AAV-3 viruses were treated with 0.1% SDS and protease k of 0.2 mg/ml at 37□°C for 3h, then was extracted with phenol/chloroform twice and extracted with chloroform once. Sodium acetate and alcohol were added to precipitate DNA. After deposition, the DNA was resuspensed by TE(PH8.0)at 95□°C for 5 min and then treated in 0.3-1.0 M NaCl at 50-60□°C for 2h until the double-strands were annealed. Qiaex IIgel extraction kit (Qiagen) was used to purify the AAV-3 DNA band of about 5 kb running out from agarose gel, then the ends of the DNA band were blunted with Klenow large segment, and after addition of a linker of XbaI Linker (dCTCTAGAG) and purification, it was cut with XbaI, loaded in XbaI site of pGEM-3zf (a product of Promega Co.) and amplified in E.Coli DH5α Max Efficiency. After the single clones were picked out and the plasmid was extracted, those clones containing complete AAV-3 genomes were screened via restrictive enzyme and the rep2 probe method. Then, the plasmid was used to transfect BHK cells and 24h later the cells were infected by HSV-1. 2 days later, small molecular weight DNAs outside the cell chromosome were extracted using Hirt method, cut with DpnI enzyme, transferred by Southern blotting and hybridized using a rep probe, and the integrity of genomes was verified by Dimer band using a monomer. After all those, pAAV3 was obtained. The pAAV3 was cut with BssHI and ApaII enzymes to recover the vector plasmid segment containing AAV-3 ITRs, then blunted with T4 DNA polymerase. Then, pSNAV-1 was cut with XhoI and

BamHI enzymes to recover the CMV-PolyA segment, then blunted with T4 DNA polymerase, and was then loaded in the vector plasmid segment containing AAV-3 ITRs, the resistance gene neo^r was cut with Bgl II and SmaI enzymes from pSV2neo of Promega Co., followed by blunting with T4 DNA polymerase and recovered, and then loaded in the vector plasmid segment containing AAV-3 ITRs. Accordingly, a recombinant plasmid pSNAV-N3 containing AAV-3 ITR elements was obtained.

Please amend the paragraph beginning at page 39, line 25 as follows:

BHK/pSNAV-GFP cells were infected with HSV1-r2c3. After cells exhibited pathology (36-72h), the cell was repeatedly frozen and thawed for 4 times. Thus, the cell lysis solution contained rAAV/r2c3-GFP and helper viruses HSV1-r2c3. A low-speed centrifugation was carried out to remove the cell debris, and then the lysis solution was treated at 56±°C for 30 min so that the helper virus HSV1-r2c3 was deactivated. Accordingly, rAAV/r2c3-GFP having the serotype of AAV-3, which could be used to infect a cultured mammal cell *in vitro* and *in vivo*, was obtained from the supernatant of the cell lysis solution.

Please amend the paragraph beginning at page 40, line 13 as follows:

pSNAV -GFPs were introduced into BHK-21 cells (purchased from ATCC and cultured in RPMI1640 medium containing 10% FBS at 37±°C) using transfecting agent Lipofectamine (produced by GIBCO BRL Co), and then the cells were selectively cultured for 10-15 days in medium with G418 of 800 ug/ml. As a result, vector cells BHK/pSNAV-GFP for mixing cell clones were obtained. Then, after being enlarged propagation, the vector cells were cultured in four 35 cm^2-square glass culture vessels; after confluent (about 8×10^7 cells), the vector cells were digested with trypsin, inoculated into roller bottles (110×288mm) followed by cultivation under a low-speed (1 rpm) rolling at 37±°C. The volume of the medium was 200 ml per roller bottle. 3 days later, the cells in the roller bottle were digested with trypsin and then introduced into 5 roller bottles for enlarged cultivation. After confluent (about 2×10^9 cells), the medium was poured out and 5-10 ml of helper viruses HSV1-r2c3(MOI = 0.5-2) was added in prior to the 1-2 hr of adsorption of the viruses at a low speed (1 rpm). Then, a serum-free 1640 medium (200 ml per roller bottle) was added in to

culture the cells at 37±°C at a low speed (1 rpm). After pathology complete, cells could be shedding easily, the bottle tops were closed tightly before being violently vortexed until all the cells attached to the bottle side were eluted into the medium. After that, the cultures in the 5 roller bottles were pooled and gathered, and after the estimation of their volumes, they were divided into erlenmeyer flasks (specification: 500 ml), 250 ml per flask, for the next purification step.

Please amend the paragraph beginning at page 41, line 5 as follows:

Continuing the above Example. 25 ml of chloroform (10:1 v/v) was added to each erlenmeyer flask, which was placed in a shaker at 37±°C for 1～1.5 hr under violent shaking before being taken out and sit at a room temperature for 10 min. DNase and RNase were then added in until the final concentration was 1 μg/ml. Then, after the mixture was blended gently until it became homogeneous, it was being digested at a room temperature for 30-60 min. Solid sodium chloride was added to a final concentration of 1 mol/L under shaking for dissolution. After that, the mixture was centrifugated at 12000 rpm at 4±°C for 15 min before the upper water phase was aspirated and the chloroform and deposits were discarded. Subsequently, PEG8000 was added in until the final concentration was 10%(w/v), and shaken to be dissolved. Then, the mixture was sit at 4±°C overnight before being centrifugated at 11000 rpm at 4±°C for 15 min. The supernatant was poured into a clean container (centrifuge tubes were turned upside down on the absorbent paper so that the supernatant could be drained to the full). After that, 5ml PBS^+ buffer was used to beat upon, elute and gather the deposits attached to the tube bottoms and tube sides of all the centrifuge tubes. Then the gathered deposits were divided into plastic centrifuge tubes (specification: 1.5 ml), 0.6 ml per tube, before being extracted by chloroform in an equal volume. The extracted mixture were centrifugated at 12000 rpm at 4±°C for 5 min before the upper water phase was carefully aspirated under antiseptic operations and then transferred into an antiseptic tube. The obtained liquid was just a condensed and purified rAAV/r2c3-GFP viral solution, of which the volume was 200 times condensed than its initial volume.

Please amend the paragraph beginning at page 42, line 5 as follows:

Continuing Example 2-7. The titer (particles/ml) of the rAAV/r2c3-GFP viruses in the purified viral solution was detected by Dot-Blotting Method using the Digoxigenin-Labeled (Boehringer Mannhein kit) GFP probe. 10 ul of the purified viral solution was diluted 1:10 with PBS^{2+} buffer. DNase and RNase were added in until the final concentration was 1 μg/ml. After the mixture was being digested at 37±°C for 1 hr and treated in boiling water-bath for 5 min, it was placed in ice bath and then diluted 1:10 with a dilution buffer before dot-blotting (1 ul/dot). Later, the membrane was baked at 120°C for 30 min, the prehybridization was conducted at 68°C for 1 hr followed by probe dot-blotting at 68°C overnight. Afterwards, the membrane was washed and developed. Results: dots 1-4 were definitely positive while dot 5 was weak positive. Supposing the sensibility of detection of DNA by Dot-Blotting Method was 10^6 molecules, it could be calculated that the viral titer=$10^{4-5}\times10^6\times10\times1000=10^{14-15}$ particles/ml.

Please amend the paragraph beginning at page 42, line 19 as follows:

At 37±°C, a 10% FBS-containing RPMI1640 medium and 5% CO_2 were used to culture HeLa cells, which were then seeded in a 24-well plate (5×10^5 cell/well). After cultivation overnight, the medium was aspirated; 10 ul of purified rAAV/r2c1-GFP viral solution was diluted 1:10 to 1 ml, then each well was added with 0.5 ml of viral solution with different dilutions and was cultured at 37±°C for 1 hr; subsequently, each well was added with 50 ul of Ad-5 (MOI=5) and 0.5 ml of medium. After 36 hr of cultivation at 37±°C, green fluorescent cells could be observed under an inverted fluorescence microscope. After the number of green cells (represented by "n", wherein $10<n<100$) in a certain well had been counted, the viral titer of rAAV/r2c3-GFP could be calculated as follows: n×Times of Dilution×1000/5=$n\times10^9\times200=2n\times10^{11}$ TU/ml. Thus, it could be estimated that the infectious titer of the rAAV/r2c3-GFP virus was between 2×10^{12} TU/ml and 2×10^{13} TU/ml.

Please amend the paragraph beginning at page 43, line 16 as follows:

Example 3-1 Construction of cos6-r2c4±△UL2

AAV-1 was used as the template, and the corresponding cap4 (AAV-4) was amplified using PCR method (primers refer to Primer Sequences 5 and 6). The reaction conditions were 30 circles of

94±°C for 30 sec, 55±°C for 30 sec and 72±°C for 3 min. A PCR segment of 2255bp, cap4 was obtained. After digesting with restrictive enzyme KpnI, cap4 was linked with a large segment to form a SSV9-cap4 plasmid, which segment was cut from pSSV9 with KpnI without the DNA segment of cap2. After digested from SSV9-cap4 plasmid with XbaI, r2c4 (of around 4536bp) was inserted into XbaI site of cos6. As a result, cos6-r2c4±△UL2 was obtained.

Please amend the paragraph beginning at page 44, line 2 as follows:

Cos6-r2c4±△UL2 was mixed at equal molar ratio with five cosmids such as cos14, cos28, cos48, cos56 and the like. Then, the cos framework was removed by PacI enzyme (no need of remove the cos framework by isolation). After that, the mixture was extracted with phenol, phenol/chloroform (1:1) and chloroform, respectively. The supernatant was aspirated and collected, and DNA was precipitated using 2.5 times anhydrous ethanol. 20 ul of lipofactamine (GIBCO BRL) and 10 ul of DNA were used to co-transfect 80% confluent BHK-21 cells (about 2×10^6 cells) according to the instructions, then homologous recombination of the 5 HSV-1 segments occurred inside the cells and accordingly HSV1-r2c4 recombinant viruses were generated, respectively. After 24h of transfection, the cell was cultured in 1640 medium containing 2% FBS at 37±°C. The medium was changed every day. After 5 days, cells began to exhibit pathology. The supernatant of the medium was collected after complete pathology followed by centrifugating at 2000 r/min for 5 min. Then, the supernatant was aliquoted and stored at -20±°C. The obtained recombinant viruses were plaque-purified twice so that pure HSV1-r2c4 recombinant viruses were prepared.

Please amend the paragraph beginning at page 44, line 17 as follows:

AAV-4 and adenovirus 5 were used to infect 293 cells. 3 days later, the cells were frozen and thawed, and then were centrifugated (5800 g) for 30 min, and purified with CsCl as described in J.V.1997, 71: 8429-8436. The above-mentioned AAV-3 viruses were treated with 0.1% SDS and protease k of 0.2 mg/ml at 37±°C for 3h, then was extracted with phenol/chloroform twice and extracted with chloroform once. Sodium acetate and alcohol were added to precipitate DNA. After deposition, the DNA was resuspensed by TE(PH8.0)at 95±°C for 5 min and then treated in 0.3-1.0 M NaCl at 50-60±°C for 2h until the double-strands was annealed. Qiaex IIgel extraction

kit(Qiagen) was used to purify the AAV-3 DNA band of about 5 kb running out from agarose gel, then the ends of the DNA band were blunted with Klenow large segment, and after addition of a linker of XbaI Linker (dCTCTAGAG) and purified, it was cut with XbaI, loaded in XbaI site of pGEM-3zf (a product of Promega Co.) and amplified in E.Coli DH5α Max Efficiency. After the single clones were picked out and the plasmid was extracted, those clones containing complete AAV-4 genomes were screened via restrictive enzyme and the rep2 probe method. Then, the plasmid was used to transfect BHK cells and 24h later the cells were infected by HSV-1. 2 days later, small molecular weight DNAs outside the cell chromosome were extracted using Hirt method, cut with DpnI enzyme, transferred by Southern blotting and hybridized using a rep probe, and the integrity of genomes was verified by Dimer band using a monomer. After all those, pAAV4 was obtained. pAAV4 was cut with Ava II and NcoI enzymes to recover the vector plasmid segment containing AAV-4 ITRs, then blunted with T4 DNA polymerase; pSNAV-GFP was cut with XhoI and BamHI enzymes to recover the CMV-PolyA segment, then blunted with T4 DNA polymerase, and was then loaded in the vector plasmid segment containing AAV-4 ITRs. Then, the resistance gene neo^r from pSV2neo of Promega Co. was cut with Bgl II and SmaI enzymes to recover the resistance gene neo^r followed by blunting with T4 DNA polymerase, and was then loaded in the vector plasmid segment containing AAV-4 ITRs. Accordingly, a recombinant plasmid pSNAV-N4 containing AAV-4 ITR elements was obtained.

Please amend the paragraph beginning at page 45, line 22 as follows:

BHK/pSNAV-GFP cells were infected with HSV1-r2c4. After cells exhibited pathology (36-72h), the cell was repeatedly frozen and thawed for 4 times. Thus, the cell lysis solution contained rAAV/r2c4-GFP and helper viruses HSV1-r2c4. A low-speed centrifugation was carried out to remove the cell debris, and then the lysis solution was treated at 56□°C for 30 min so that the helper virus HSV1-r2c4 was deactivated. Accordingly, rAAV/r2c4-GFP having the serotype of AAV-4, which could be used to infect a cultured mammal cell *in vitro/in vivo*, was obtained from the supernatant of the cell lysis solution.

Please amend the paragraph beginning at page 46, line 10 as follows:

pSNAV -GFPs were introduced into BHK-21 cells (purchased from ATCC and cultured in RPMI1640 medium containing 10% FBS at 37±°C) using transfecting agent Lipofectamine (produced by GIBCO BRL Co), and then the cells were selectively cultured for 10-15 days in medium with G418 of 800 ug/ml. As a result, vector cells BHK/pSNAV-GFP for mixing cell clones were obtained. Then, after being enlarged propagation, the vector cells were cultured in four 35 cm^2-square glass culture vessels; after confluent (about 8×10^7 cells), the vector cells were digested with trypsin, inoculated into roller bottles (110×288mm) followed by cultivation under a low-speed (1 rpm) rolling at 37±°C. The volume of the medium was 200 ml per roller bottle. 3 days later, the cells in the roller bottle were digested with trypsin and then introduced into 5 roller bottles for enlarged cultivation. After confluent (about 2×10^9 cells), the medium was poured out and 5-10 ml of helper viruses HSV1-r2c4(MOI = 0.5-2) was added in prior to the 1-2 hr of adsorption of the viruses at a low speed (1 rpm). Then, a serum-free 1640 medium (200 ml per roller bottle) was added in to culture the cells at 37±°C at a low speed (1 rpm). After pathology complete, cells could be shedding easily, the bottle tops were closed tightly before being violently vortexed until all the cells attached to the bottle side were eluted into the medium by shaken acutely. After that, the cultures in the 5 roller bottles were pooled and gathered, and after the estimation of their volumes, they were divided into erlenmeyer flasks (specification: 500 ml), 250 ml per flask, for the next purification step.

Please amend the paragraph beginning at page 47, line 1 as follows:

Continuing the above Example. 25 ml of chloroform (10:1 v/v) was added to each erlenmeyer flask, which was placed in a shaker at 37±°C for 1～1.5 hr under violent shaking before being taken out and sit at a room temperature for 10 min. DNase and RNase were then added in until the final concentration was 1 μg/ml. Then, after the mixture was blended gently until it became homogeneous, it was being digested at a room temperature for 30-60 min. Solid sodium chloride was added to a final concentration of 1 mol/L under shaking for dissolution. After that, the mixture was centrifugated at 12000 rpm at 4±°C for 15 min before the upper water phase was aspirated and the chloroform and deposits were discarded. Subsequently, PEG8000 was added in until the final

concentration was 10% (w/v), and shaken to be dissolved. Then, the mixture was sit at 4□°C overnight before being centrifugated at 11000 rpm at 4□°C for 15 min. The supernatant was poured into a clean container (centrifuge tubes were turned upside down on the absorbent paper so that the supernatant could be drained to the full). After that, 5ml PBS^+ buffer was used to beat upon, elute and gather the deposits attached to the tube bottoms and tube sides of all the centrifuge tubes. Then the gathered deposits were divided into plastic centrifuge tubes (specification: 1.5 ml), 0.6 ml per tube, before being extracted by chloroform in an equal volume. The extracted mixture were centrifugated at 12000 rpm at 4□°C for 5 min before the upper water phase was carefully aspirated via an antiseptic operation and then transferred into an antiseptic tube. The obtained liquid was just a condensed and purified rAAV/r2c4-GFP viral solution, of which the volume was 200 times condensed than its initial volume.

Please amend the paragraph beginning at page 48, line 1 as follows:

Continuing Example 3-7. The titer (particles/ml) of the rAAV/r2c4-GFP viruses in the purified viral solution was detected by Dot-Blotting Method using the Digoxigenin-Labeled (Boehringer Mannhein kit) GFP probe. 10 ul of the purified viral solution was diluted 1:10 with PBS^{2+} buffer. DNase and RNase were added in until the final concentration was 1 μg/ml. After the mixture was being digested at 37□°C for 1 hr and treated in boiling water-bath for 5 min, it was placed in ice bath and then diluted 1:10 with a dilution buffer before dot-blotting (1 ul/dot). Later, the membrane was baked at 120°C for 30 min, the prehybridization was conducted at 68°C for 1 hr followed by probe dot-blotting at 68°C overnight. Afterwards, the membrane was washed and developed. Results: dots 1-4 were definitely positive while dot 5 was weak positive. Supposing the sensibility of detection of DNA by Dot-Blotting Method was 10^6 molecules, it could be calculated that the viral titer=$10^{4-5}\times10^6\times10\times1000=10^{14-15}$ particles/ml.

Please amend the paragraph beginning at page 48, line 14 as follows:

At 37□°C, a 10% FBS-containing RPMI1640 medium and 5% CO_2 were used to culture HeLa cells, which were then seeded in a 24-well plate (5×10^5 cell/well). After cultivation overnight, the medium was absorbed out; 10 ul of purified rAAV/r2c4-GFP viral solution was diluted 1:10 to 1 ml,

then each well was added with 0.5 ml of viral solution with different dilutions and was cultured at 37±°C for 1 hr; subsequently, each well was added with 50 ul of Ad-5(MOI=5) and 0.5 ml of medium. After 36 hr of cultivation at 37±°C, green fluorescent cells could be observed under an inverted fluorescence microscope. After the number of green cells (represented by "n", wherein $10<n<100$) in a certain well had been counted, the viral titer of rAAV/r2c4-GFP could be calculated as follows: n×Times of Dilution×1000/5 = $n\times10^{9}\times200=2n\times10^{11}$ TU/ml. Thus, it could be estimated that the infectious titer of the rAAV/r2c4-GFP virus was between 2×10^{12} TU/ml and 2×10^{13} TU/ml.

Please amend the paragraph beginning at page 49, line 11 as follows:

Example 4-1 Construction of cos6-r2c5±△UL2

AAV-5 was used as the template, and the corresponding cap5 (AAV-5) was amplified using PCR method (primers refer to Primer Sequences 7 and 8). The reaction conditions were 30 circles of 94±°C for 30 sec, 55±°C for 30 sec and 72±°C for 3 min. A PCR segment of 2170bp, cap5 was obtained. After double digesting with restrictive enzymes BamHI and XbaI, cap1 was ligated with rep 2 of 860bp, which was digested from pSSV9 with BamHI and XbaI. The ligation product was inserted into XbaI site of plasmid pGEM-p3zf(+) (Promega Co.) to form p3zf-r2c5 plasmid. After digested from p3zf-r2c5 plasmid with XbaI, r2c5 (of around 4314bp) was inserted into XbaI site of cos6. As a result, cos6-r2c5±△UL2 was obtained.

Please amend the paragraph beginning at page 49, line 26 as follows:

Cos6-r2c5±△UL2 was mixed at equal molar ratio with five cosmids such as cos14, cos28, cos48, cos56 and the like. Then, the cos framework was removed by PacI enzyme (no need of remove the cos framework by isolation). After that, the mixture was extracted with phenol, phenol/chloroform (1:1) and chloroform, respectively. The supernatant was aspirated and collected, and DNA was precipitated using 2.5 times anhydrous ethanol. 20 ul of lipofactamine (GIBCO BRL) and 10 ul of DNA were used to co-transfect 80% confluent BHK-21 cells (about 2×10^{6} cells) according to the instructions, then homologous recombination of the 5 HSV-1 segments occurred inside the cells and accordingly HSV1-r2c5 recombinant viruses were generated, respectively. After 24h of transfection, the cell was cultured in 1640 medium containing 2% FBS at 37±°C. The

medium was changed every day. After 5 days, cells began to exhibit pathology. The supernatant of the medium was collected after complete pathology followed by centrifugating at 2000 r/min for 5 min. Then, the supernatant was aliquoted and stored at -20□°C. The obtained recombinant viruses were plaque-purified twice so that pure HSV1-r2c5 recombinant viruses were prepared.

Please amend the paragraph beginning at page 50, line 13 as follows:

AAV-5 and adenovirus 5 were used to infect 293 cells. 3 days later, the cells were frozen and thawed, and then were centrifugated (5800 g) for 30 min, and purified with CsCl as described in J.V.1997, 71: 8429-8436. The abovementioned AAV-4 viruses were treated with 0.1% SDS and protease k of 0.2 mg/ml at 37□°C for 3h, then was extracted with phenol/chloroform twice and extracted with chloroform once. Sodium acetate and alcohol were added to precipitate DNA. After deposition, the DNA was resuspensed by TE(PH8.0)at 95□°C for 5 min and then treated in 0.3-1.0 M NaCl at 50-60□°C for 2h until the double-strands was annealed. Qiaex IIgel extraction kit (Qiagen) was used to purify the AAV-4 DNA band of about 5 kb running out from agarose gel, then the ends of the DNA band were blunted with Klenow large segment, and after addition of a linker of XbaI Linker (dCTCTAGAG) and purified, it was cut with XbaI, loaded in XbaI site of pGEM-3zf (a product of Promega Co.) and amplified in E.Coli DH5α Max Efficiency. After the single clones were picked out and the plasmid was extracted, those clones containing complete AAV-4 genomes were screened via restrictive enzyme and the rep2 probe method. Then, the plasmid was used to transfect BHK cells and 24h later the cells were transfected by HSV-1. 2 days later, small molecular weight DNAs outside the cell chromosome were extracted using Hirt method, cut with DpnI enzyme, transferred by Southern blotting and hybridized using a rep probe, and the integrity of genomes was verified by Dimer band using a monomer. After all those, pAAV5 was obtained. The pAAV5 was cut with BssH II and MseI double digestion to recover the vector plasmid segment containing AAV-5 ITRs, then blunted with T4 DNA polymerase; pSNAV-GFP was cut with XhoI and BamHI enzyme to recover the CMV-PolyA segment, then blunted with T4 DNA polymerase after was recycled, and was then loaded in the vector plasmid segment containing AAV-5 ITRs. Then, the resistance gene neo^r from pSV2neo of Promega Co. was cut with Bgl II and SmaI enzyme to recover the resistance gene neo^r followed by blunting with T4 DNA polymerase, and was then loaded in the vector plasmid

segment containing AAV-5 ITRs. Accordingly, a recombinant plasmid pSNAV-N5 containing AAV-5 ITR elements was obtained.

Please amend the paragraph beginning at page 51, line 18 as follows:

BHK/pSNAV-GFP cells were infected with HSV1-r2c5. After cells exhibited pathology (36-72h), the cell was repeatedly frozen and thawed for 4 times. Thus, the cell lysis solution contained rAAV/r2c5-GFP and helper viruses HSV1-r2c5. A low-speed centrifugation was carried out to remove the cell debris, and then the lysis solution was treated at 56□°C for 30 min so that the helper virus HSV1-r2c5 was deactivated. Accordingly, rAAV/r2c5-GFP having the serotype of AAV-5, which could be used to infect a cultured mammal cell *in vitro*/*in vivo*, was obtained from the supernatant of the cell lysis solution.

Please amend the paragraph beginning at page 52, line 6 as follows:

pSNAV -GFPs were introduced into BHK-21 cells (bought from ATCC and cultured in RPMI1640 medium containing 10% FBS at 37□°C) using transfecting agent Lipofectamine (produced by GIBCO BRL Co), and then the cells were selectively cultured for 10-15 days in medium with G418 of 800 ug/ml. As a result, vector cells BHK/pSNAV-GFP for mixing cell clones were obtained. Then, after being enlarged propagation, the vector cells were cultured in four 35 cm^2-square glass culture vessels; after confluent (about 8×10^7 cells), the vector cells were digested with trypsin, inoculated into roller bottles (110×288mm) followed by cultivation under a low-speed (1 rpm) rolling at 37□°C. The volume of the medium was 200 ml per roller bottle. 3 days later, the cells in the roller bottle were digested with trypsin and then introduced into 5 roller bottles for enlarged cultivation. After confluent (about 2×10^9 cells), the medium was poured out and 5-10 ml of helper viruses HSV1-r2c5(MOI = 0.5-2) was added in prior to the 1-2 hr of adsorption of the viruses at a low speed (1 rpm). Then, a serum-free 1640 medium (200 ml per roller bottle) was added in to culture the cells at 37□°C at a low speed (1 rpm). After pathology complete, cells could be shedding easily, the bottle tops were closed tightly before being violently vortexed until all the cells attached to the bottle side were eluted into the medium. After that, the cultures in the 5 roller bottles were

pooled and gathered, and after the estimation of their volumes, they were divided into erlenmeyer flasks (specification: 500 ml), 250 ml per flask, for the next step—purification.

Please amend the paragraph beginning at page 52, line 25 as follows:

Continuing the above Example. 25 ml of chloroform (10:1 v/v) was added to each erlenmeyer flask, which was placed in a shaker at 37±°C for 1～1.5 hr under violent shaking before being taken out and sit at a room temperature for 10 min. DNase and RNase were then added in until the final concentration was 1 μg/ml. Then, after the mixture was blended gently until it became homogeneous, it was being digested at a room temperature for 30-60 min. Solid sodium chloride was added to a final concentration of 1 mol/L under shaking for dissolution. After that, the mixture was centrifugated at 12000 rpm at 4±°C for 15 min before the upper water phase was removed and the chloroform and deposits were discarded. Subsequently, PEG8000 was added in until the final concentration was 10%(w/v), and shaken to be dissolved. Then, the mixture was sit at 4±°C overnight before being centrifugated at 11000 rpm at 4±°C for 15 min. The supernatant was poured into a clean container (centrifuge tubes were turned upside down on the absorbent paper so that the supernatant could be drained to the full). After that, 5ml PBS^+ buffer was used to beat upon, elute and gather the deposits attached to the tube bottoms and tube sides of all the centrifuge tubes. Then the gathered deposits were aliquoted into plastic centrifuge tubes (specification: 1.5 ml), 0.6 ml per tube, before being extracted by chloroform in an equal volume. The extracted mixture were centrifugated at 12000 rpm at 4±°C for 5 min before the upper water phase was carefully aspirated via an antiseptic operation and then transferred into an antiseptic tube. The obtained liquid was just a condensed and purified rAAV/r2c5-GFP viral solution, of which the volume was 200 times condensed than its initial volume.

Please amend the paragraph beginning at page 53, line 25 as follows:

Continuing Example 4-7. The titer (particles/ml) of the rAAV/r2c5-GFP viruses in the purified viral solution was detected by Dot-Blotting Method using the Digoxigenin-Labeled (Boehringer Mannhein kit) GFP probe. 10 ul of the purified viral solution was diluted 1:10 with

PBS^{2+}buffer. DNase and RNase were added in until the final concentration was 1 μg/ml. After the mixture was being digested at 37□°C for 1 hr and treated in boiling water-bath for 5 min, it was placed in ice bath and then diluted 1:10 with a dilution buffer before dot-blotting (1 ul/dot). Later, the membrane was baked at 120°C for 30 min, the prehybridization was conducted at 68°C for 1 hr followed by probe dot-blotting at 68°C overnight. Afterwards, the membrane was washed and developed. Results: dots 1-4 were definitely positive while dot 5 was weak positive. Supposing the sensibility of detection of DNA by Dot-Blotting Method was 10^6 molecules, it could be calculated that the viral titer=$10^{4-5}\times10^6\times10\times1000=10^{14-15}$ particles/ml.

Please amend the paragraph beginning at page 54, line 11 as follows:

At 37□°C, a 10% FBS-containing RPMI1640 medium and 5% CO_2 were used to culture HeLa cells, which were than seeded in a 24-well plate (5×10^5 cell/well). After cultivation overnight, the medium was absorbed out; 10 ul of purified rAAV/r2c5-GFP viral solution was diluted 1:10 to 1 ml, then each well was added with 0.5 ml of viral solution with different dilutions and was cultured at 37□°C for 1 hr; subsequently, each well was added with 50 ul of Ad-5(MOI=5) and 0.5 ml of medium. After 36 hr of cultivation at 37□°C, green fluorescent cells could be observed under an inverted fluorescence microscope. After the number of green cells (represented by "n", wherein 10<n<100) in a certain well had been counted, the viral titer of rAAV/r2c5-GFP could be calculated as follows: n×Times of Dilution×1000/5=$n\times10^9\times200=2n\times10^{11}$ TU/ml. Thus, it could be estimated that the infectious titer of the rAAV/r2c5-GFP virus was between 2×10^{12} TU/ml and 2×10^{13} TU/ml.

Please amend the paragraph beginning at page 55, line 8 as follows:

Example 5-1 Construction of cos6-r2c6□△UL2

AAV-1 was used as the template, and the corresponding cap6 (AAV-6) was amplified using PCR method (primers refer to Primer Sequences 9 and 10). The reaction conditions were 30 circulations of 94□°C for 30 sec, 55□°C for 30 sec and 72□°C for 3 min. A PCR segment of 2210bp, cap6 was obtained. After double digesting with restrictive enzymes KpnI and XbaI, cap6 was linked with rep 2 of 1721bp, which was digested from pSSV9 with KpnI and XbaI. The ligation

product was inserted into XbaI site of plasmid pGEM-p3zf(+) (Promega Co.) to form p3zf-r2c6 plasmid. After digested from p3zf-r2c6 plasmid with XbaI, r2c6 (of around 4239 bp) was inserted into XbaI site of cos6. As a result, cos6-r2c6□△UL2 was obtained.

Please amend the paragraph beginning at page 55, line 23 as follows:

Cos6-r2c6□△UL2 was mixed at equal molar ratio with five cosmids such as cos14, cos28, cos48, cos56 and the like. Then, the cos framework was removed by PacI enzyme (no need of remove the cos framework by isolation). After that, the mixture was extracted with phenol, phenol/chloroform (1:1) and chloroform, respectively. The supernatant was absorbed and collected, and DNA was precipitated using 2.5 times anhydrous ethanol. 20 ul of lipofactamine (GIBCO BRL) and 10 ul of DNA were used to co-transfect 80% confluent BHK-21 cells (about 2×10^6 cells) according to the instructions, then homologous recombination of the 5 HSV-1 segments occurred inside the cells and accordingly HSV1-r2c6 recombinant viruses were generated, respectively. After 24h of transfection, the cell was cultured in 1640 medium containing 2% FBS at 37□°C. The medium was changed every day. After 5 days, cells began to exhibit pathology. The supernatant of the medium was collected after complete pathology followed by centrifugating at 2000 r/min for 5 min. Then, the supernatant was aliquoted and stored at -20□°C. The obtained recombinant viruses were plaque-purified twice so that pure HSV1-r2c6 recombinant viruses were prepared.

Please amend the paragraph beginning at page 56, line 10 as follows:

AAV-6 and adenovirus 5 were used to infect 293 cells. 3 days later, the cells were frozen and thawed, and then were centrifugated (5800 g) for 30 min, and purified with CsCl as described in J.V.1997, 71: 8429-8436. The above-mentioned AAV-6 viruses were treated with 0.1% SDS and protease k of 0.2 mg/ml at 37□°C for 3h, then was extracted with phenol/chloroform twice and extracted with chloroform once. Sodium acetate and alcohol were added to precipitate DNA. After deposition, the DNA was resuspensed by TE(PH8.0)at 95□°C for 5 min and then treated in 0.3-1.0 M NaCl at 50-60□°C for 2h until the double-strands was annealed. Qiaex IIgel extraction kit (Qiagen) was used to purify the AAV-6 DNA band of about 5 kb running out from agarose gel, then the ends of the DNA band were flanked with Klenow large segment, and after addition of a linker of

XbaI Linker (dCTCTAGAG) and purification, it was cut with XbaI, loaded in XbaI site of pGEM-3zf (a product of Promega Co.) and amplified in E.Coli DH5α Max Efficiency. After the single clones were picked out and the plasmid was extracted, those clones containing complete AAV-6 genomes were screened via restrictive enzyme and the rep2 probe method. Then, the plasmid was used to transfect BHK cells and 24h later the cells were transfected by HSV-1. 2 days later, small molecular weight DNAs outside the cell chromosome were extracted using Hirt method, cut with DpnI enzyme, transferred by Southern blotting and hybridized using a rep probe, and the integrity of genomes was verified by Dimer band using a monomer. After all those, pAAV6 was obtained. The pAAV6 was cut with PmacI and BstE II double digestion to recover the vector plasmid segment containing AAV-6 ITRs, then blunted with T4 DNA polymerase; pSNAV-GFP was cut with XhoI and BamHI enzyme to recover the CMV-PolyA segment, then blunted with T4 DNA polymerase, and was then loaded in the vector plasmid segment containing AAV-6 ITRs. Then, the resistance gene neo^r was cut with Bgl II and SmaI enzyme from pSV2neo of Promega Co., followed by blunted with T4 DNA polymerase, and was then loaded in the vector plasmid segment containing AAV-6 ITRs. Accordingly, a recombinant plasmid pSNAV-N6 containing AAV-6 ITR elements was obtained.

Please amend the paragraph beginning at page 57, line 15 as follows:

BHK/pSNAV-GFP cells were infected with HSV1-r2c6. After cells exhibited pathology (36-72h), the cell was repeatedly frozen and thawed for 4 times. Thus, the cell lysis solution contained rAAV/r2c6-GFP and helper viruses HSV1-r2c6. A low-speed centrifugation was carried out to remove the cell debris, and then the lysis solution was treated at 56□°C for 30 min so that the helper virus HSV1-r2c6 was deactivated. Accordingly, rAAV/r2c6-GFP having the serotype of AAV-6, which could be used to infect a cultured mammal cell *in vitro*/*in vivo*, was obtained from the supernatant of the cell lysis solution.

Please amend the paragraph beginning at page 58, line 3 as follows:

pSNAV -GFPs were introduced into BHK-21 cells (bought from ATCC and cultured in RPMI1640 medium containing 10% FBS at 37□°C) using transfecting agent Lipofectamine

(produced by GIBCO BRL Co), and then the cells were selectively cultured for 10-15 days in medium with G418 of 800 ug/ml. As a result, vector cells BHK/pSNAV-GFP for mixing cell clones were obtained. Then, after being enlarged propagation, the vector cells were cultured in four 35 cm^2-square glass culture vessels; after confluent (about 8×10^7 cells), the vector cells were digested with trypsin, inoculated into roller bottles (110×288mm) followed by cultivation under a low-speed (1 rpm) rolling at 37±°C. The volume of the medium was 200 ml per roller bottle. 3 days later, the cells in the roller bottle were digested with trypsin and then introduced into 5 roller bottles for enlarged cultivation. After confluent (about 2×10^9 cells), the medium was poured out and 5-10 ml of helper viruses HSV1-r2c6(MOI = 0.5-2) was added in prior to the 1-2 hr of adsorption of the viruses at a low speed (1 rpm). Then, a serum-free 1640 medium (200 ml per roller bottle) was added in to culture the cells at 37±°C at a low speed (1 rpm). After pathology complete, cells could be shedding easily, the bottle tops were closed tightly before being violently vortexed until all the cells attached to the bottle side were eluted into the medium. After that, the cultures in the 5 roller bottles were collected and gathered, and after the estimation of their volumes, they were divided into erlenmeyer flasks (specification: 500 ml), 250 ml per flask, for the next purification step.

Please amend the paragraph beginning at page 58, line 22 as follows:

Continuing the above Example. 25 ml of chloroform (10:1 v/v) was added to each erlenmeyer flask, which was placed in a shaker at 37±°C for 1～1.5 hr under violent shaking before being taken out and sit at a room temperature for 10 min. DNase and RNase were then added in until the final concentration was 1 μg/ml. Then, after the mixture was blended gently until it became homogeneous, it was being digested at a room temperature for 30-60 min. Solid sodium chloride was added to a final concentration was 1 mol/L under shaking for dissolution. After that, the mixture was centrifugated at 12000 rpm at 4±°C for 15 min before the upper water phase was removed and the chloroform and deposits were discarded. Subsequently, PEG8000 was added in until the final concentration was 10% (w/v), and shaken to be dissolved. Then, the mixture was sit at 4±°C overnight before being centrifugated at 11000 rpm at 4±°C for 15 min. The supernatant was poured into a clean container (centrifuge tubes were turned upside down on the absorbent paper so that the supernatant could be drained to the full). After that, 5ml PBS^+ buffer was used to beat upon, elute

and gather the deposits attached to the tube bottoms and tube sides of all the centrifuge tubes. Then the gathered deposits were divided into plastic centrifuge tubes (specification: 1.5 ml), 0.6 ml per tube, before being extracted by chloroform in an equal volume. The extracted mixture were centrifugated at 12000 rpm at 4±°C for 5 min before the upper water phase was carefully aspirated via an antiseptic operation and then transferred into an antiseptic tube. The obtained liquid was just a condensed and purified rAAV/r2c6-GFP viral solution, of which the volume was 200 times condensed than its initial volume.

Please amend the paragraph beginning at page 59, line 22 as follows:

Continuing Example 5-7. The titer (particles/ml) of the rAAV/r2c6-GFP viruses in the purified viral solution was detected by Dot-Blotting Method using the Digoxigenin-Labeled (Boehringer Mannhein kit) GFP probe. 10 ul of the purified viral solution was diluted 1:10 with PBS^{2+} buffer. DNase and RNase were added in until the final concentration was 1 μg/ml. After the mixture was being digested at 37±°C for 1 hr and treated in boiling water-bath for 5 min, it was placed in ice bath and then diluted 1:10 with a dilution buffer before dot-blotting (1 ul/dot). Later, the membrane was baked at 120°C for 30 min, the prehybridization was conducted at 68°C for 1 hr followed by probe dot-blotting at 68°C overnight. Afterwards, the membrane was washed and developed. Results: dots 1-4 were definitely positive while dot 5 was weak positive. Supposing the sensibility of detection of DNA by Dot-Blotting Method was 10^6 molecules, it could be calculated that the viral titer=$10^{4-5}\times10^6\times10\times1000=10^{14-15}$ particles/ml.

Please amend the paragraph beginning at page 60, line 8 as follows:

At 37±°C, a 10% FBS-containing RPMI1640 medium and 5% CO_2 were used to culture HeLa cells, which were than seeded in a 24-well plate (5×10^5 cell/well). After cultivation overnight, the medium was absorbed out; 10 ul of purified rAAV/r2c6-GFP viral solution was diluted 1:10 to 1 ml, then each well was added with 0.5 ml of viral solution with different dilutions and was cultured at 37±°C for 1 hr; subsequently, each well was added with 50 ul of Ad-5(MOI=5) and 0.5 ml of medium. After 36 hr of cultivation at 37±°C, green fluorescent cells could be observed under an inverted fluorescence microscope. After the number of green cells (represented by "n", wherein

10<n<100) in a certain well had been counted, the viral titer of rAAV/r2c6-GFP could be calculated as follows: n×Times of Dilution×1000/5 = n×10^9×200 = 2n×10^{11} TU/ml. Thus, it could be estimated that the infectious titer of the rAAV/r2c6-GFP virus was between 2×10^{12} TU/ml and 2×10^{13} TU/ml.